http:// ijp.mums.ac.ir
Original Article (Pages: 10099-10107)

Acute Hepatorenal Dose Dependent Toxicity of Teucrium Polium Hydro Alcoholic Extract in Rat

Tayyeb Ghasemi[1, 2], Mansoor Keshavarz[1, 2], *Mohsen Parviz [1, 2]

[1]Basic Electrophysiology Research Centre, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran.

[2]Department of Physiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background

Based on vast usage of teucrium polium (TP) in alternative medicine of developing countries for treatment of ailments in children and adults, this study is designed to examine acute hydro alcoholic extract of TP effects in different doses on rat liver and kidney functions and tissue structures.

Materials and Methods

Animals were given daily intraperitoneal (i.p.) injection of TP at 3, 10, 30, 100, and 200 mg/kg or equal volume of normal saline for a week. One-hour postprandial blood glucose at day 1 and day 7, liver enzymes, serum creatinine (Cr), blood urea nitrogen (BUN) and hepatorenal tissues were examined at the end of the study. Animal body weights were also measured on a daily basis.

Results

TP at 3, 10, 30 and 100 mg/kg body weight didnot affect functional and structural characteristics in rat liver and kidney tissues compared with control animals. However, at high 200 mg/kg dose, it provoked liver and kidney tissue damages together with significant rise in aspartate aminotransferase ($p<0.001$), alanine aminotransferase ($p=0.001$), Cr ($p=0.001$), and BUN ($p=0.001$). Animal body weight in each group under TP pretreatment protocol unchanged during the study except at high 200 mg/kg which showed a significant weight loss ($p<0.001$).

Conclusion

TP detrimental health effects especially on liver and kidney tissues are frequently overemphasized, but in a dose-dependent manner. However, we also believe TP has potential medical benefits and can find a way to the medical arena if thorough conducted researches can determine its toxic components and isolate appropriate derivatives for ensuing use.

Key Words: Iranian Traditional Medicine, Liver Toxicity, Kidney Toxicity, Teucrium Polium.

*Please cite this article as: Ghasemi T, Keshavarz M, Parviz M. Acute Hepatorenal Dose Dependent Toxicity of Teucrium Polium Hydro Alcoholic Extract in Rat. Int J Pediatr 2019; 7(9): 10099-10107. DOI: **10.22038/ijp.2019.41222.3475**

Corresponding Author:

Mohsen Parviz (MD), Associate Professor of Physiology, Department of Physiology, School of Medicine, Tehran University of Medical Sciences, school of medicine, Tehran, Iran, P.O Box: 1417613151. Tel (Fax): +982166419484.

Email: parvizmo@tums.ac.ir

Received date: Feb.15, 2019; Accepted date: Aug.12, 2019

1- INTRODUCTION

Teucrium polium (TP) is a medicinal herb from *Lamiaceae* family that is commonly found in Mediterranean regions like Spain, Algeria, Tunisia, Turkey, Syria, Iraq and Iran. Due to its beneficial antispasmodic and gastrointestinal effects, it is broadly used, especially in developing countries for abdominal pain in children and also in adults for other therapeutic goals (1-3). Its use as a medicinal herb dates back to Hippocrates, Dioscorides, Palin and Galen. In Iranian traditional medicine, TP or Kalpureh in Persian language is recommended for use in diabetes, diarrhea, abdominal tension, pain, inflammation, bacterial infection, convulsion and dementia. This herb has been used for over 2000 years as diuretic, sweat gland activator, tonic substance, and antispasmodic and bile stimulator in traditional medicine (4).

Moreover, it has also been used to treat obesity (5), acetaminophen toxicity and cognitive disturbances (6-10). TP is a potent antioxidant and anticholine-esterase substance (11). In addition, its anti-inflammatory and anti-apoptotic effects in liver cells have been proved. Liver cells treated with TP extract are resistent to oxygen free radicals effects and inflammatory responses (12). Intramuscular injection of TP can regenerate cortical and hippocampal neural circuits and increase neural survival (13).

Despite broad evidence implying TP therapeutic effects in traditional medicine, there are striking arguments over its hepatorenal side effects which have not been resolved (14). Yet, impressive findings can be found in the literature during a web search which point to the old and controversial debate on the issue (15). In this study, which was designed as preliminary, we first tested the hepatorenal safety of the hydro-alcoholic extract of the plant in different low dose ranges and proved its sound administration in our setting.

2- MATERIALS AND METHODS

2-1. Study design and setting

This is a basic experimental study designed and carried out from early January till mid-July of 2018 in Physiology Department of Tehran University of Medical Sciences. Animals' care and handling were carried out in accordance with Tehran University of Medical Sciences Ethics Committee measures and those of National Institute of Health (NIH).

2-2. Plant extract

TP Lamiaceae was collected from Alborz mountain region near Karaj, Iran during spring and dried in shade at room temperature. Herbarium samples were kept on record No. PMP-387 in pharmaceutical faculty, taxonomy department of Tehran University of Medical Sciences. The aerial parts of TP plant were first dried in the shadow and ground with a mortar to form a powder. Then 2000 g of the powdered material was macerated with ethanol and water (75/25 v/v) to get the hydro-alcoholic extract for 72 h in room ambient temperature. Maceration was repeated for another 48 and 24 h again for evaporation and then filtered with Whatman paper. The final crude extract was put into the oven at 55 ^{o}C for 36 h before storing. Doses of 3, 10, 30, 100, and 200 mg/kg of TP were prepared in normal saline and intraperitoneally (i.p.) injected to check the hepatorenal effects and get the optimal dose for ensuing research.

2-3. Animals

Thirty male Wistar rats aged 10-12 weeks (weighing 230-240 g) were kept in plexiglas cages in Tehran University of Medical Sciences, at constant temperature and humidity with free access to water and standard chow in a 12-h light dark cycle

during the study. To test the acute toxicity of TP, animals underwent daily i.p. injection of 3, 10, 30, 100, and 200 mg/kg of hydro-alcoholic extract of TP for a week and equal volume of 2 ml of normal saline in control group.

2-4. Liver and kidney toxicity assay

Animals were euthanized under deep anesthesia with high doses of ketamine and xylazine at the end of the study. Blood samples were taken from decapitated animals for analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Cr), Alkaline Phosphatase (ALKP) with spectrophotometer technique. Tail origin one-hour post prandial glucose level at day 1 and day 7 in the end were measured with the same lab method. Total body weight was also monitored on daily basis during the experiment.

2-5. Histological exam

Histological study was also performed on liver and kidney tissues with hematoxylin and eosin (H&E) stain technique. In brief, prepared dehydrated paraffinized liver and kidney tissues were sectioned in 5 to 10 µm and deparaffinized, hydrated, stained with hematoxylin and eosin solutions and dehydrated in ascending alcohol solutions. Sections were mounted with coverslip on glass slide for final drying stage (16).

2-6. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM) and compared by one-way ANOVA and two-way ANOVA with Tukey test in Graphpad Prism statistical software version 6.0. P-value less than 0.05 was considered statistically significant.

3- RESULTS

Daily i.p. administration of 3, 10, 30 and 100 mg/kg hydro-alcoholic extract of TP for one week did not statistically change ALT, AST and ALKP compared with control ones (**Table.1**). Kidney function parameters of BUN and Cr followed the same pattern ($p>0.05$) (**Table. 2**). At 200 mg/kg dose however, significantly increased ALT ($p<0.001$), AST ($p<0.001$), BUN ($p<0.0001$), and Cr ($p<0.0001$) were found compared with control and TP pretreated rats at different doses (**Tables 1 and 2**). ALKP level, however, did not statistically change at different doses ($p>0.05$).

One-hour post prandial blood glucose level at day 1 and day 7 was not statistically altered in TP pretreated rats at different doses compared with rats in control group ($p>0.05$) (**Table.3**). Body weight in different TP pretreated doses was unchanged except at high 200 mg/kg dose which showed a significant reduction ($p<0.0001$) compared with control animals (**Table.4**).

Table-1: Liver function serologic parameters in control and different TP received groups.

Groups	ALT	P- value	AST	P- value	ALKP	P- value
	Mean± SEM		Mean± SEM		Mean± SEM	
Control	58.8± 3.8	Ref.	160.8± 11.3	Ref.	406± 8.7	Ref.
3 mg/kg	66.6± 3.8	0.7258	207± 14.2	0.5248	444± 21.5	0.9901
10 mg/kg	62± 1.9	0.9918	203.8± 7.1	0.5985	582± 53	0.0879
30 mg/kg	65.6± 3.1	0.8221	190.8± 7.9	0.8661	556± 46	0.1923
100 mg/kg	72.2± 5.0	0.1933	198.2 ± 13.3	0.7251	595± 33	0.0573
200 mg/kg	89.8± 5.1	<0.0001	317± 38.9	< 0.0001	566± 67.8	0.1443

SEM: Standard error of mean; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALKP: Alkaline phosphatase; Ref.: Reference category.

Table-2: Kidney function serologic parameters in control and different TP received groups.

Groups	BUN	P- value	Cr	P- value
	Mean± SEM		Mean± SEM	
Control	23.33± 1.5	Ref.	0.51± 0.02	Ref.
3 mg/kg	19.6± 1.3	0.9390	0.57± 0.02	0.9993
10 mg/kg	24.6± 1.6	0.9992	0.54± 0.01	> 0.9999
30 mg/kg	24.8± 1.2	0.9984	0.52± 0.01	> 0.9999
100 mg/kg	25.8± 1.7	0.9847	0.53± 0.01	> 0.9999
200 mg/kg	80.6± 6.1	< 0.0001	1.36± 0.28	0.0004

SEM: Standard error of mean; BUN: Blood urea nitrogen; Cr: Creatinine; Ref.: Reference category.

Table-3: One-hour post prandial blood glucose level at day 1 and day 7 in control and TP pretreated groups.

Groups	Day 1	P- value	Day 7	P- value
	Mean± SEM		Mean± SEM	
Control	108.8± 7.5	Ref.	108.8± 7.5	Ref.
3 mg/kg	99.4± 7.8	0.8714	96.6± 2.0	0.3586
10 mg/kg	100.6± 6.2	0.9229	99.6± 1.4	0.6515
30 mg/kg	101.4± 3.6	0.9487	101± 2.5	0.7852
100 mg/kg	101.2± 6.3	0.9429	99.4± 3.3	0.6312
200 mg/kg	105.2± 2.6	0.9980	108.6± 5.3	> 0.9999

SEM: Standard error of mean; Ref.: Reference category.

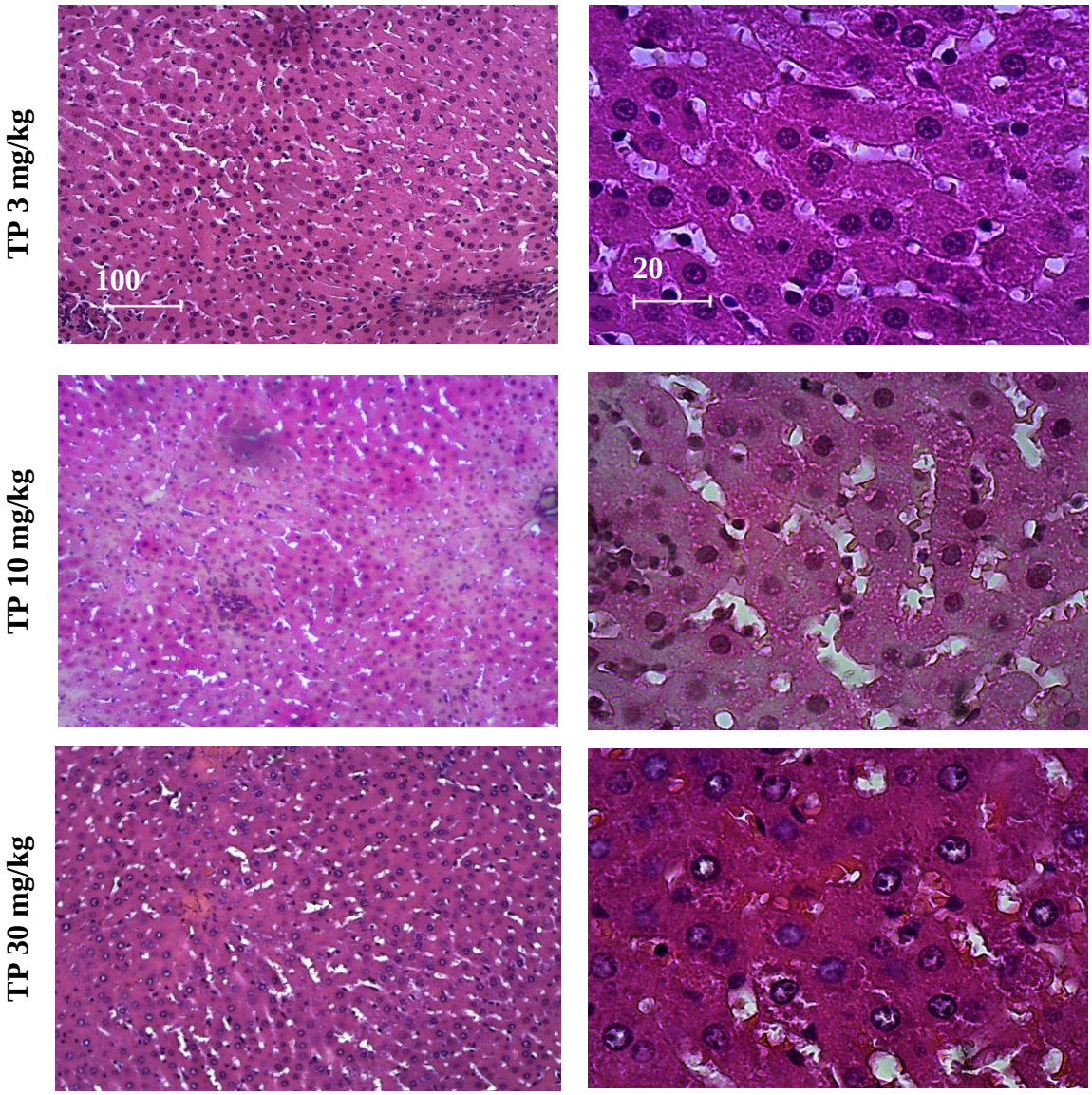
Table-4: Body weight comparison at day 1 and day 7 in control and TP pretreated groups.

Groups	Day 1	Day 7	P- value
	Mean± SEM	Mean± SEM	
Control	230± 7.5	226± 8.4	> 0.9999
3 mg/kg	225± 5.7	229± 6.4	0.9250
10 mg/kg	228± 9.3	235± 8.1	0.5333
30 mg/kg	233± 6.2	239± 7.0	> 0.9999
100 mg/kg	233± 9	226± 8.9	0.4966
200 mg/kg	233± 9.3	189± 7.6	< 0.0001

SEM: Standard error of mean.

Liver tissues were intact in different TP pretreated doses of 3, 10, 30 and 100 mg/kg. In these groups, polygonal multinucleated hepatocytes can be seen. Sinusoidal space was normal. Necrosis or apoptosis were absent. Kupffer cells sufficient in number can also be seen in sinusoidal spaces. Fat-like accumulation is distinctive in some cells. Collagen content of the liver tissue was normal with no sign of fibrosis. Cells with bright pink cytoplasmic color and notable membrane can be identified. Moreover, meticulously arranged lobular structure with normal size was noted. Portal triads in normal size were seen in vicinity of hepatocytes. Most sinusoids have normal space. In TP 200 pretreated rats, however, advanced inflammation and necrosis in vast areas of hepatic tissue are noted. Foamy hepatocytes with pale color cytoplasm and unmarked membrane can be seen. Moreover, totally disorganized lobular structure is seen in most areas. Portal triads

became smaller and gathered near lobular structure. Sinusoids were larger than normal due to hepatocyte degeneration in their walls (**Figure.1**). Kidney tissues are normal in TP pretreated rats compared with normal control animals. In cortical layer, primary glomeruli, proximal and distal convoluted tubules have cuboidal cell with demarcated border of cell membrane and nuclei. In glomeruli part and macula densa, basement membrane has a normal integrity. In some glomeruli, however, lymphocytic infiltration is noted. In medullary layer, loop of Henle and collecting ducts have normal cell membrane, though, in some tubules, some cellular membranes were damaged with drug dose escalation. Afferent and efferent arterioles with peritubular capillary network were meticulously arranged with normal endothelium near renal lobes and lobules (**Figure.2**). In medullary layer, loop of Henle and collecting ducts with normal cell membrane are noted. Damaged membrane tubules can be seen in some areas with drug dose escalation. See afferent and efferent arterioles with peritubular capillary network meticulously arranged with normal endothelium near renal lobes and lobules.



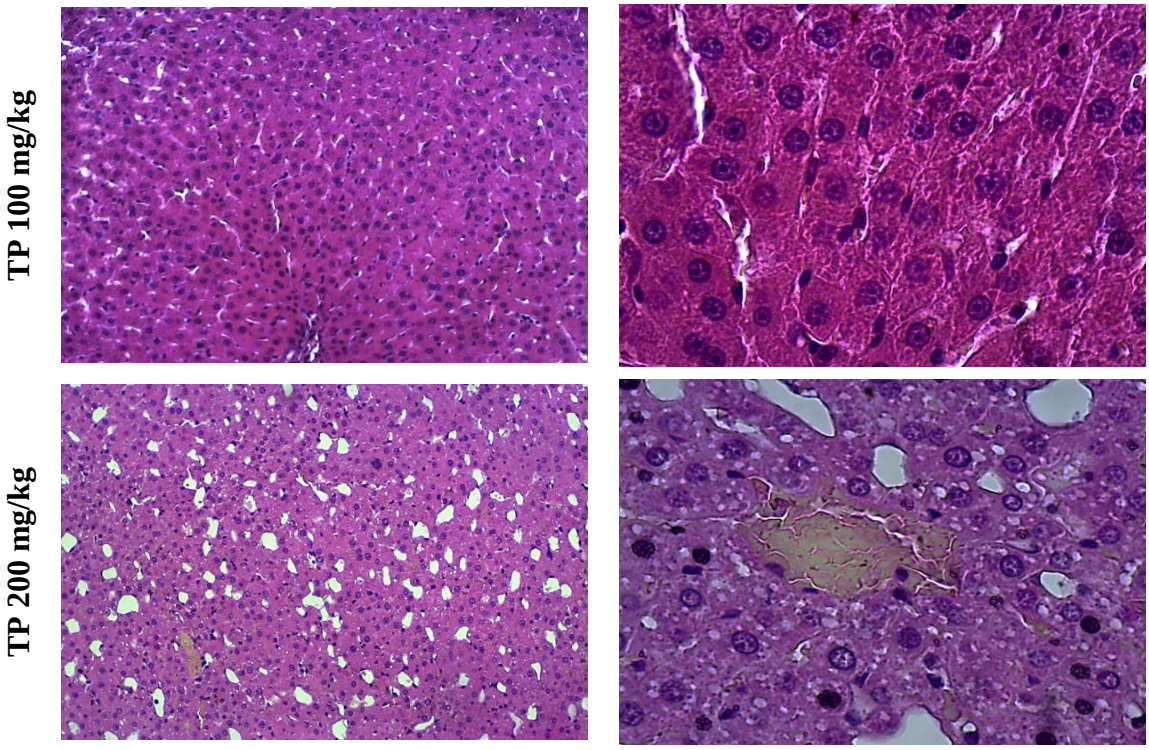


Fig.1: Liver tissues in teucrium polium (TP) pretreated animals at 3, 10, 30 and 100 mg/kg doses. Images were taken with x40 and x100 magnification on right and on left sides, respectively. Note intact liver tissues with polygonal multinucleated hepatocytes, bright pink cytoplasmic color and notable membrane with normal sinusoidal space surrounded by Kupffer cells. Fat-like accumulation in some cells can be seen. Also, note normal collagen content, meticulously arranged lobular structure, normal sized portal triads near hepatocytes with no trace of necrosis or apoptosis. In TP200 pretreated rats, however, advanced inflammation and necrosis in vast areas of hepatic tissue are seen. Foamy hepatocytes with pale color cytoplasm and unmarked membrane can be seen. Totally disorganized lobular structure with adjacent small portal triads and large sinusoids due to hepatocyte degeneration in their walls are remarkable.

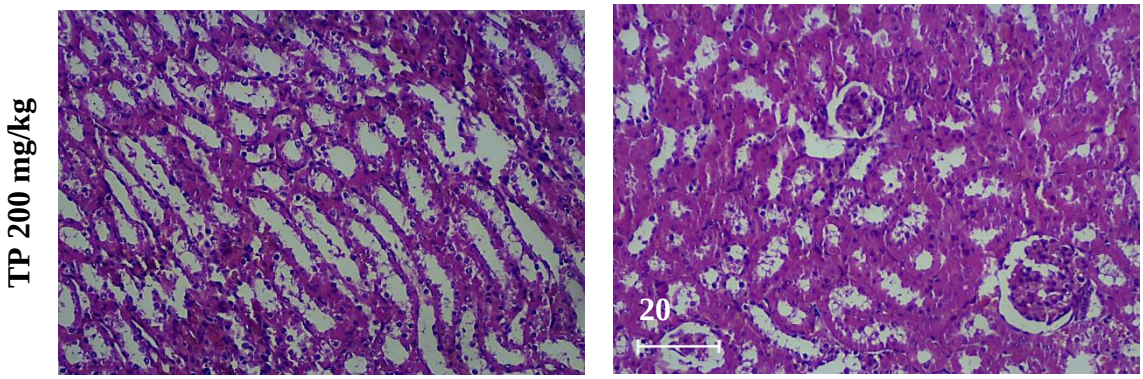


Fig.2: Kidney tissue of cortical (right) and medullary (left) segments in teucrium polium (TP) pretreated animals at 200 mg/kg doses. Images were taken with x100 magnification. In cortical layer, note normal cuboidal cells with demarcated membrane and nuclei in primary glomeruli, proximal and distal convoluted tubule. Basement membrane with normal integrity can be seen in glomeruli and macula densa. Lymphocytic infiltration can be found in some areas.

4- DISCUSSION

This study is designed to examine acute hydro alcoholic extract of TP effects in different doses on rat liver and kidney functions and tissue structures. Our findings showed TP at low doses of 3, 10, 30 and 100 mg/kg did not statistically change liver and kidney functional markers and tissue microscopic structural integrity compared with control animals. However, at 200 mg/kg high dose, it significantly increased AST, ALT, BUN and Cr and caused both structural and functional changes parallel to each other. TP controversial safety reputation has been shown in different studies (17-19). Despite repeated reports of TP hazardous effects and its hepatorenal toxicity with unknown mechanisms especially at high doses or with long-term use and in some cases even liver transplant, its use is still continued for diabetes and infantile colic treatment (3, 20-22). In the present study, acute administration of TP at 3, 10, 30, 100 and 200 mg/kg did not alter glucose level compared with the controls despite mounting documented evidence of TP antidiabetic properties in hyperglycemic (2, 23), and euglycemic (24) states.

This discrepancy may come from different animal and plant species, fasting and normal food regiment, TP extract preparation method and the time lapse between its administration and blood glucose measurement. Yet, there are some reports indicating TP glucose neutral effect in euglycemic states (25). Hepatorenal toxicity of TP has always been a topic of scientific debate (17, 26), but now its beneficial health effects are gradually reappeared with new insight (9, 27). This is due to our increasing knowledge on different TP chemotypes and toxicity (14), new chemical tools and techniques, dose-dependent nature of TP properties, route of TP administration, acute and chronic course of treatment, diverse plant species, and disparity between animal and human studies. This study is a pilot preliminary one and has its own special drawbacks. One is the lack of blood samples in different time interval for better comparisons during the course of study, but that would be inevitable due to anemia concern. Chronic assessment is also obligatory for toxicity study and is strongly recommended for thorough conclusion.

5- CONCLUSION

TP extract toxicity during acute or chronic models in research settings or human use indicate crude material toxicity. Thus, its use as a hypoglycemic agent or in other therapies of traditional medicine practices should definitely be discouraged until final complementary results have been presented.

6- ABBREVIATION

SEM: Standard error of mean
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
ALKP: Alkaline phosphatase
Ref.: Reference category
BUN: Blood urea nitrogen
Cr: Creatinine.

7- CONFLICT OF INTEREST: None.

8- AKNOWLEDGMENT

This research has been supported by Tehran University of Medical Sciences & Health Services grant (No: 96-01-139-3439).

9- REFERENCES

1. Sezer, R.G. and A. Bozaykut, Pediatric hepatotoxicity associated with polygermander (Teucrium polium). Clinical Toxicology, 2012; 50(2): 153-53.

2. Shahraki MR, Arab MR, Mirimokaddam E, Palan MJ. The effect of Teucrium polium (Calpoureh) on liver function, serum lipids and glucose in diabetic male rats. Iran Biomed J, 2007; 11(1): 65-68.

3. Mattéi A, Rucay P, Samuel D, Feray C, Reynes M, Bismuth H. Liver transplantation for severe acute liver failure after herbal medicine:(Teucrium polium) administration. Journal of hepatology, 1995; 22(5): 597.

4. Galati EM, Mondello MR, D'Aquino A, Miceli N, Sanogo R, Tzakou O, et al. Effects of Teucrium divaricatum Heldr. ssp. divaricatum decoction on experimental ulcer in rats. J Ethnopharmacol, 2000; 72(1-2): 337-42.

5. M.-S.Suleiman, A.-S Abdul-Ghanib, S.Al-Khalil, R.Amin. Effect of Teucrium polium boiled leaf extract on intestinal motility and blood pressure. J Ethnopharmacol, 1988; 22(1): 11-6.

6. Rasekh H.R, M.J. Khoshnood-Mansourkhani, M. Kamalinejad. Hypolipidemic effects of Teucrium polium in rats. Fitoterapia, 2001; 72(8): 937-9.

7. Abdollahi M, H. Karimpour HR. Monsef-Esfehani, Antinociceptive effects of Teucrium polium L. total extract and essential oil in mouse writhing test. Pharmacological Research, 2003; 48(1): 31-5.

8. Couladis M, Tzakou O, Verykokidou E, Harvala C. Screening of some Greek aromatic plants for antioxidant activity. Phytotherapy research, 2003; 17(2): 194-95.

9. Forouzandeh H, Azemi ME, Rashidi I, Goudarzi M, Kalantari H. Study of the protective effect of Teucrium polium L. extract on acetaminophen-induced hepatotoxicity in mice. Iranian journal of pharmaceutical research: IJPR, 2013; 12(1): 123.

10. Hasanein P, S. Shahidi. Preventive effect of Teucrium polium on learning and memory deficits in diabetic rats. Medical science monitor: international medical journal of experimental and clinical research, 2012; 18(1): BR41.

11. Muamer Dizdar, Danijela Vidic, Franc Požgan, Bogdan Štefane, Milka Maksimović. Acetylcholinesterase inhibitory, antioxidant and phytochemical properties of selected medicinal plants of the Lamiaceae family. Molecules, 2014; 19(1): 767-82.

12. Aghazadeh S, R. Yazdanparast. Inhibition of JNK along with activation of ERK1/2 MAPK pathways improve steatohepatitis among the rats. Clinical nutrition, 2010; 29(3): 381-85.

13. V. A. Chavushyan, K. V. Simonyan, H. M. Galstyan. In vivo electrophysiological study of effects of acute and chronic systemic application of hydroponic Teucrium polium extract. Морфологія, 2012; 6(2): 58-69.

14. Alessandro Venditti, Claudio Frezza, Eugenia Trancanella, Seyed Majid Majd Zadeh, Sebastiano Foddai, Fabio Sciubba, et al. A new natural neo-clerodane from Teucrium polium L. collected in Northern Iran. Industrial crops and products, 2017; 97: 632-38.

15. Baali N, Belloum Z, Baali S, Chabi B, Pessemesse L, Fouret G, et al., Protective Activity of Total Polyphenols from Genista quadriflora Munby and Teucrium polium geyrii Maire in Acetaminophen-Induced Hepatotoxicity in Rats. Nutrients, 2016; 8(4): 193.

16. Sheehan D, B. Hrapchak. Theory andPractice ofHistotechnology. CV Mosby Co., St. Louis, 1980. p.48.

17. Larrey D. Hepatotoxicity of herbal remedies. Journal of Hepatology, 1997; 26: 47-51.

18. Bahramikia, S. and R. Yazdanparast, Phytochemistry and medicinal properties of Teucrium polium L. (Lamiaceae). Phytother Res, 2012; 26(11): 1581-93.

19. Khadige A, Keshavarz Z, Mojab F, Majd HA. The effect of Teucrium polium on the duration of menstrual bleeding: A triple-blind placebo-controlled clinical trial. Electron Physician, 2017; 9(9): 5233-36.

20. Polymeros D, D. Kamberoglou, V. Tzias. Acute cholestatic hepatitis caused by Teucrium polium (golden germander) with transient appearance of antimitochondrial antibody. J Clin Gastroenterol, 2002; 34(1): 100-1.

21. Baradaran A, Madihi Y, Merrikhi A, Rafieian-Kopaei M, Nematbakhsh M, Asgari A, et al. Nephrotoxicity of hydroalcoholic extract ofTeucrium polium in Wistar rats.

Nephrotoxicity of hydroalcoholic extract of Teucrium polium in Wistar rats. Pak J Med Sci 2013; 29(1)Suppl:329-33.

22. Sezer RG, A. Bozaykut. Pediatric hepatotoxicity associated with polygermander (teucrium polium). Clin Toxicol (Phila): 2012; 50(2): 153.

23. Mousavi SE, Shahriari A, Ahangarpour A, Vatanpour H, Jolodar A. Effects of Teucrium polium Ethyl acetate Extract on Serum, Liver and Muscle Triglyceride Content of Sucrose-InducedInsulin Resistance in Rat. Iran J Pharm Res, 2012; 11(1): 347-55.

24. Gharaibeh M.N., H.H. Elayan, A.S. Salhab. Hypoglycemic effects of Teucrium polium. J Ethnopharmacol, 1988; 24(1):93-9.

25. Mehmet Iriadam,Davut Musa,Hatice Gümüflhan, Füsun Baba. Effects of two Turkish medicinal plants Artemisia herba-alba and Teucrium polium on blood glucose levels and other biochemical parameters in rabbits. J Cell Mol Biol, 2006; 5(1): 19-24.

26. Al-Ashban R., D. Barrett, A. Shah. Effects of chronic treatment with ethanolic extract of Teucrium polium in mice. Journal of herbs, spices & medicinal plants, 2006; 11(4): 27-36.

27. Rahmouni F, Hamdaoui L, Badraoui R, Rebai T. Protective effects of Teucrium polium aqueous extract and ascorbic acid on hematological and some biochemical parameters against carbon tetrachloride (CCl4) induced toxicity in rats. Biomedicine andPharmacotherapy, 2017; 91: 43-8.